

# COAXIAL TURBULENT JET MIXER FOR CONTROLLED SYNTHESIS OF NANOPARTICLES

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## ABSTRACT

Development of methods for high-throughput production of nanoparticles (NPs) in a reproducible and controlled manner is important for the translation of NPs to the clinic. We report a simple and versatile coaxial turbulent jet mixer platform for high-throughput preparation of various functional NPs in a reproducible and controllable manner. With a production rate sufficiently high for *in vivo* studies, clinical trials, and industrial scale production, this approach has the potential to improve the homogeneity and provide better quality control in the translation of nanomedicines to the clinic.

**KEYWORDS:** Nanoparticles, mixer, turbulent jet, nanomedicine

## INTRODUCTION

NPs have shown great promise for the diagnosis and therapy of major diseases such as cancer, cardiovascular, and infectious diseases.[1,2] Use of microfluidics for synthesis of these NPs has been of great interest due to controllability and reproducibility in their physicochemical properties.[1,3] However, there are intrinsic limitations in conventional polydimethylsiloxane (PDMS) microfluidic systems for the synthesis of NPs. First, expensive equipments and clean room facility are required to prepare a master mold for the replication of PDMS microchannels, which make difficult to utilize the PDMS microfluidic systems in normal biomedical research lab.[3] Second, only a handful of organic solvents are compatible with conventional PDMS microfluidic systems,[4] which hinders PDMS microfluidic systems from serving as versatile platforms for synthesis of various types of NPs. Third, productivity of microfluidic systems is lower than that of batch reactors due to low flow rates, making it challenging to produce large quantities of NPs required for *in vivo* or clinical studies.[1,2] Here, we report a novel coaxial turbulent jet mixer capable of synthesizing various types of NPs with high production rates suitable for *in vivo* studies and clinical trials, while maintaining the advantages controllability and reproducibility of PDMS microfluidic systems.

## THEORY

In the coaxial turbulent jet mixer, the inner flow stream containing raw materials is mixed with an outer stream of non-solvent by turbulent jet flow for generation of uniform NPs by rapid solvent exchange method called nanoprecipitation (Figure 1a).[5,6] The mixing of the inner and outer streams was visualized using phenolphthalein, a pH indicator (Inset of Figure 1a and Figure 1b). As mixing occurred in the coaxial turbulent jet mixer, the color of liquid jet changed from pink to colorless, because the acidic outer solution neutralized or acidified the basic inner solution.

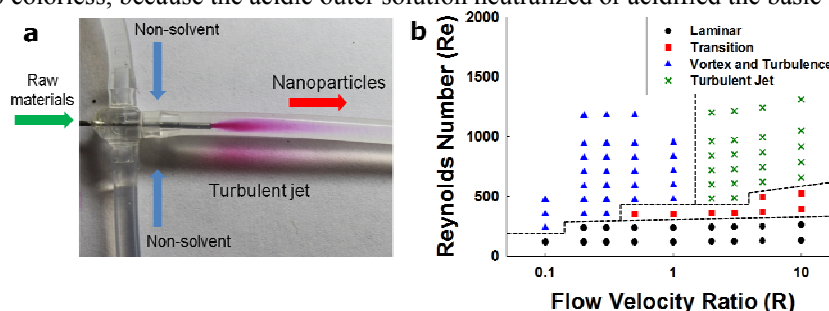


Figure 1. (a) Schematic illustration of a coaxial turbulent jet mixer for large-scale synthesis of NPs. The inset shows the top view of the turbulent jet. (b) Phase diagram of jet flow regime in terms of  $Re$  and  $R$ .

Two dimensionless parameters, flow velocity ratio ( $R$ , defined as the ratio of the average velocity of the inner fluid to the average velocity of the outer fluid) and Reynolds number ( $Re$ ),[7] were used to classify the mixing behavior into laminar, transition, vortex and turbulence, and turbulent jet regimes as summarized in the phase diagram (Figure 1b). In laminar flow regime, the inner flow is focused by outer flow and stable stratified flow is maintained. Because slow diffusional mixing is dominant in the laminar flow regime, the fluid is not completely mixed. In the transition regime, the flow is unstable and switches from laminar flow to turbulent flow, and vice versa. In the vortex and turbulence regime, a

micro-vortex is generated in outer flow and turbulence is developed at the tip of focused inner flow. In the turbulent jet regime, inner stream forms a turbulent jet, leading to rapid mixing of fluid across the entire cross-section of the tube. This regime is therefore desired for the formation of NPs.

In case of vortex and turbulence regime and turbulent jet regimes, we could estimate the mixing length ( $L$ ) as a function of  $Re$  and  $R$ .  $L$  was defined as the length at which the gray value is 90 % of the intensity difference between the completely mixed region far downstream along the centerline and the tip of the syringe needle (Figure 2a). In case of laminar regime,  $L$  could not be estimated because complete mixing was not achieved. In case of transition regime,  $L$  also could not be estimated because the flow was unstable. To estimate the mixing timescale,  $\tau_{mix}$ , in a simple way, we assume that the mixed fluid flow at  $u_{avg}$  throughout the mixing process so that  $\tau_{mix} = L / u_{avg}$ . In the turbulent jet regime, the  $\tau_{mix}$  was tunable in the range of 1 - 40 ms by changing the  $Re$  since mixing is expedited as  $Re$  increases (Figure 2b).

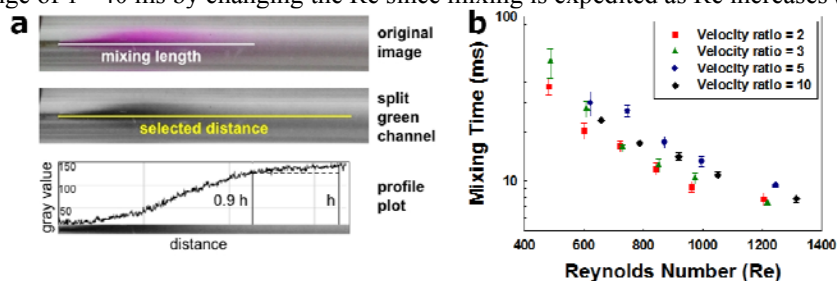


Figure 2. (a) The image processing method for the estimation of mixing length ( $L$ ).  $L$  is defined as the distance at which the gray value of the RGB green channel is 90% of its maximum value ( $h$ ). (b) Mixing time ( $\tau_{mix}$ ) as a function of  $Re$  when inner flow velocity is faster than outer flow velocity (i.e.,  $R > 1$ ).

## EXPERIMENTAL

The coaxial turbulent jet mixer was prepared by inserting a syringe needle into a tee union tube fitting. A wide choice of standard fittings, syringe needles, and materials can be used to construct the coaxial turbulent jet mixer. For demonstration, we used PTFE fittings and tubing that are compatible with a variety of solvents. The tee union tube fitting made of PTFE (Plasmatech Co.) was drilled by a 0.025 inch diameter drill bit (Drill bit city). 23 G blunt needle (Strategic applications Inc.) was inserted through the drilled hole and fixed by optical adhesive (NOA81, Norland products) under UV light. By using connector and adaptor (IDEX health & science), PTFE tubing (Plasmatech Co.) was connected to the tee union tube fitting. For the preparation of NPs, precursor solution (10 mg/mL poly(lactide-*co*-glycolide)-*b*-polyethyleneglycol (PLGA-PEG) in acetonitrile for PLGA-PEG NPs and 5 mmol/L dimyristoylphosphatidylcholine, cholesterol, and dihexadecyl phosphate in a molar ratio of 5 : 4 : 1 in isopropyl alcohol for lipid vesicles) and non-solvent (deionized water for PLGA-PEG NPs and phosphate buffered saline for lipid vesicles) were used as inner and outer stream, respectively. During the NP synthesis, the flow rates were controlled by syringe pumps (Harvard Apparatus). In case of bulk synthesis, 100  $\mu$ L of precursor solution was mixed drop-wise with 1 mL of non-solvent for about 2 h under magnetic stirring. The resulting NP suspensions were purified by ultrafiltration using Amicon Ultracel 100 K membrane filters.

## RESULTS AND DISCUSSION

The coaxial turbulent jet mixer is a versatile platform which can synthesize various types of NPs by rapid nanoprecipitation in a controlled and high-throughput manner. While conventional PDMS microfluidic devices are not compatible with many organic solvents,[4] various types of NP precursors can be adopted when the coaxial turbulent jet mixer is made of PTFE tee union and Teflon tubes due to their excellent compatibility with various organic solvents. As a proof of concept, we demonstrated the preparation of different NPs including PLGA-PEG NPs (Figure 3a), lipid vesicles (Figure 3b), iron oxide NPs (data not shown), and polystyrene NPs (data not shown) by using the coaxial turbulent jet mixer.

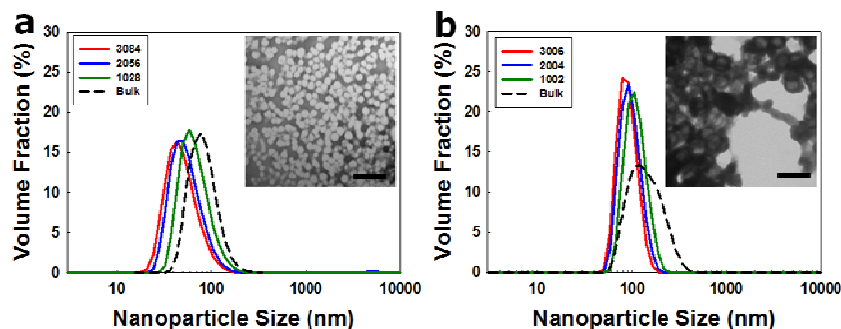


Figure 3. The size distribution by volume fraction of (a) PLGA-PEG NPs and (b) lipid vesicles prepared using coaxial turbulent jet mixer at different  $Re$  and using bulk synthesis method. The insets show TEM images of the NPs. Scale bars are 200 nm.

In case of PLGA-PEG NPs, the production rate in a coaxial turbulent jet mixer was estimated based on the total flow rate and concentration of PLGA-PEG polymer in inner flow stream (Table 1). A production rate up to 1.875 g/min was achieved when the coaxial turbulent jet mixer was operated at high Re and high concentration (*i.e.*, 50 mg/mL). Here, the coaxial turbulent jet mixer was operated in the turbulent jet regime with fixed ratio of inner stream to outer stream (*i.e.*, 1 : 10 in volumetric flow rate ratio).

Reynolds number	1028	2056	3084
Total flowrate	137.5 mL/min	275 mL/min	412.5 mL/min
Production rate (10 mg/mL)	0.125 g/min	0.25 g/min	0.375 g/min
Production rate (50 mg/mL)	0.625 g/min	1.25 g/min	1.875 g/min

Table 1. Total flow rate in a coaxial turbulent jet mixer and production rate of PLGA-PEG NPs with different polymeric precursor concentrations (*i.e.*, 10 mg/mL and 50 mg/mL) at three distinct Re (*i.e.*, 1028, 2056, and 3084).

The synthesized NPs were uniform in size as confirmed in both dynamic light scattering data (Figure 3) and TEM images (inset of Figure 3). The size distributions of NPs prepared by coaxial turbulent jet mixer are more uniform compared to that of NPs prepared by conventional bulk synthesis methods (Figure 3). Using the coaxial turbulent jet mixer, the size of the NPs could be precisely controlled simply by changing Re for fixed NP precursor solution (Figure 3), since  $\tau_{mix}$  is precisely controllable by changing Re (Figure 2b). NPs obtained using the coaxial turbulent jet mixer are smaller than those synthesized by the bulk synthesis method because the  $\tau_{mix}$  is on the order of or smaller than the characteristic aggregation time scale ( $\tau_{agg}$ ) (Figure 3).[5,6] In the current design of coaxial turbulent jet mixer, the size of NPs could be controlled precisely and reproducibly in the range of 50 - 100 nm by simply changing Re, when PLGA<sub>95K</sub>-PEG<sub>5K</sub> at a concentration of 10 mg/mL were used as polymeric precursors (Figure 3a). Similar to the case of PLGA-PEG NPs (Figure 3a), the size of lipid vesicles (Figure 3b), iron oxide NPs (data not shown), and polystyrene NPs (data not shown) could be controlled precisely and reproducibly by simply changing Re. The influence of  $\tau_{mix}$  on the size of NPs disappears at a certain Re, suggesting that these NPs have reached the size limit for the NP precursor solution.[5]

## CONCLUSION

We developed a simple and versatile coaxial turbulent jet mixer that can synthesize various type of NPs with high production rates, while maintaining the advantages of better homogeneity and control due to rapid mixing that are normally accessible only by using specialized microfabricated devices. The coaxial turbulent jet mixer can be fabricated within 30 min without requiring specialized equipment, micro-fabrication facilities, or specialized skills, making it easily accessible to nanomedicine researchers. The coaxial turbulent jet mixer can be used as a versatile platform for reproducible and controlled synthesis of NPs with high-throughput manner. In case of PLGA-PEG NPs, we could achieve the production rate up to 1.875 g/min, which is equivalent to 2.7 kg/day. Considering that the production rates typically required in clinical studies and industrial scale productions of NPs are order of 0.1 kg/day and 1 kg/day, respectively, it is noteworthy that a single coaxial turbulent jet mixer can meet both requirements. The technology can potentially expedite the translation of personalized nanomedicines to the clinic and industrial scale production of NPs.

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